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Meinardi, [No Value]; Henkens, CMA; de Kam, PJ; van der Meer, Jan

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# Acquired APC Resistance in Neurosurgical Patients May Not Be a Risk Factor for Postoperative Deep Vein Thrombosis

\*Johan R. Meinardi, M.D., \*Cilia M. A. Henkens, M.D., Ph.D., †Pieter-Jan de Kam, M.Sc., and  
\*Jan van der Meer, M.D., Ph.D.

*\*Division of Hemostasis, Thrombosis and Rheology, University Hospital, Groningen, and †Trial Coordination Centre, University Hospital, Groningen, The Netherlands*

**Summary:** Acquired resistance to activated protein C has been reported during oral contraception and pregnancy. Its thrombogenic potential was studied in 41 neurosurgical patients who were enrolled in the placebo group of a thromboprophylaxis trial. Normalized activated protein C sensitivity ratio (nAPC-SR), clotting activity of factors V and VIII, and levels of protein C antigen were measured prior to and at days 3 and 7 after surgery. Bilateral venography was done in all patients at days 8–10 to demonstrate deep vein thrombosis. A lowered nAPC-SR was found in 76% (baseline), 80% (day 3), and 88% (day 7) of patients. It was inversely related to factor VIII clotting activity ( $p = .0003$ ) and protein C antigen, ( $p =$

.02). Deep vein thrombosis was demonstrated in 30% of patients with a normal nAPC-SR and in 23% of patients with a lowered nAPC-SR. Pulmonary embolism was not observed. Multivariate analysis did not identify a lowered nAPC-SR as a thrombotic risk factor, in contrast with gender (women,  $p = .02$ ) and Quetelet index ( $\geq 25 \text{ kg/m}^2$ ,  $p = .006$ ). Our data provide no evidence that acquired activated protein C resistance, frequently found in neurosurgical patients, contributes to their high risk of postoperative deep vein thrombosis. **Key Words:** Thromboembolism—Blood coagulation disorders—APC resistance—Factor V—Neurosurgery.

Hereditary resistance to activated protein C (APC) is the most common genetic defect associated with an increased risk of venous thromboembolism (1,2). In the vast majority of affected individuals, APC resistance results from a single point mutation of the blood coagulation factor V gene (factor V:Q<sup>506</sup> mutation or factor V Leiden), which renders the activated form of factor V less susceptible to proteolytic inactivation by APC (3). Hereditary APC resistance is commonly demonstrated by a lowered ratio of the activated partial thromboplastin time (aPTT), which is measured in the presence and absence of APC, respectively (APC ratio) (4). It is most reliably diagnosed by DNA analysis. Recently, a lowered APC ratio, not due to the factor V:Q<sup>506</sup> mutation, has been reported in women using oral contraceptives and in pregnant women (5–7). Another study showed that women who used third-generation oral contraceptives were significantly less sensitive to APC than those using second-generation oral contraceptives (8). These observations suggest acquired APC resistance. Its association with known thrombotic risk factors has even been inter-

preted as the causal explanation of thrombosis in these conditions (8,9).

Because surgery is another established risk factor for venous thromboembolism, the question arises whether acquired APC resistance contributes to the development of postoperative venous thromboembolism. We conducted a retrospective analysis of data derived from a prospective, randomized thromboprophylaxis trial to estimate the incidence of acquired APC resistance and its relation to postoperative venous thromboembolism.

## PATIENTS AND METHODS

### Patients

The study contained all patients in our center who were enrolled in a double-blind, randomized, placebo-controlled, multicenter trial that was designed to assess the efficacy and safety of the low molecular weight heparin nadroparin calcium (Fraxiparin<sup>TM</sup>, Sanofi, Paris, France) in the prevention of venous thromboembolism in neurosurgical patients (10). Consecutive patients (age  $\geq 18$  years) were included who were undergoing craniotomy or spinal column surgery because of tumor, subarachnoid bleeding, or clipping of a single aneurysm. Written informed consent was obtained. The patients were randomly assigned to subcutaneous treatment with

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Address correspondence and reprint requests to Dr. J. R. Meinardi, Division of Haemostasis, Thrombosis and Rheology, University Hospital Groningen, Hanzplein 1, 9713 GZ Groningen, The Netherlands.

nadoparin calcium or placebo from the first postoperative day. All patients wore elastic compression stockings from shortly before surgery until discharge. Patients who underwent craniotomy were treated with dexamethasone (16 mg daily) from 2 days before surgery until the second postoperative day, when the dose was tapered. Hydrocortisone was given to patients with a pituitary tumor from the evening before operation. Prior to discontinuation of the trial medication (8 to 10 days after surgery) bilateral venography was performed. It was performed earlier if clinical suspicion of deep vein thrombosis (DVT) was found and B-mode compression ultrasonography was abnormal. Ventilation-perfusion lung scanning was performed if pulmonary embolism was clinically suspected.

### Laboratory tests

Coagulation tests were performed on plasma samples collected 1 day before surgery and on days 3 and 7 after surgery. Nine-tenths volume of whole blood was mixed with 1/10th volume of trisodiumcitrate. Platelet-free plasma was prepared by centrifugation for 10 minutes at 4,000 rpm, followed by 5 minutes at 14,000 rpm in a microcentrifuge, and it was frozen at  $-80^{\circ}\text{C}$  until assays were performed. The APC ratio was measured using the commercially available Coatest<sup>TM</sup> APC resistance kit (Chromogenix AB, Mölndal, Sweden) and expressed as normalized APC-sensitivity ratio (nAPC-SR) (4). The normal range of nAPC-SR was 0.82–1.22, defined as mean  $\pm$  2 SD in healthy volunteers. Clotting activity of factor V (factor V:C) and factor VIII (factor VIII:C) were measured by one-stage clotting assays on a KC10A Amelung Coagulometer (Amelung GmbH, Lemgo, Germany) and levels of protein C antigen (PC) by enzyme-linked immunosorbent assay (ELISA) (reagents obtained from DAKO, Glostrup, Denmark). Deficient plasmas for factor V:C and factor VIII:C assays were delivered by Organon Teknika Corp., Durham, NC, U.S.A. Pooled normal plasma for assays was obtained from 65 healthy volunteers, 51 men and 14 women, mean age 37 years (range 22–63 years). They all had a negative personal and family history of either thrombosis or bleeding tendency, did not use oral contraceptives or other drugs, and were not pregnant within the last 3 months.

### Statistics

Patients with established venous thromboembolism were compared with patients in whom venous thromboembolism could not be demonstrated. Baseline characteristics were summarized to evaluate differences between the groups. Normally distributed continuous variables were analyzed with the Student's *t* test and presented by mean and standard deviation. Skewed distributed continuous variables were analyzed using the Wilcoxon's two-sample test and presented as median and

range. Categorical variables were analyzed by Fisher exact test or the chi-square test, when appropriate. To evaluate changes in time, analysis for repeated measurements was performed using a random effects model (11). Logistic regression analysis was used to identify covariables related to the occurrence of venous thromboembolism. The results are expressed by odds ratios. Univariate and multiple regression analysis were performed to assess the influence of clinical and laboratory characteristics on nAPC-SR. Regression coefficients are presented. All *p* values are two sided and a *p* < .05 was considered statistically significant. Analysis was performed using SAS software, version 6.12 (SAS-Institute Inc., Cary, North Carolina, U.S.A.).

## RESULTS

The present study included 61 of the 120 patients in our center who received placebo. Of this group, 20 patients were excluded from analysis because a bilateral venogram could not be obtained or was not evaluable (12 patients) or plasma samples were not available (8 patients). The remaining 41 patients (22 men, 19 women) had a mean age of 48 years (range 20–73 years). None of the women were pregnant, one woman used an oral contraceptive. A histologic diagnosis was obtained in all patients with a tumor. Deep vein thrombosis was demonstrated by venography in 10 (24.4%) of 41 evaluable patients. Five patients had proximal DVT, one patient had bilateral proximal DVT. None of the patients revealed clinical suspicion of DVT or pulmonary embolism. Baseline characteristics are summarized in Table 1. Patients who developed DVT were significantly more often female (*p* = .03) and had a significantly higher Quetelet index (*p* = .01). Age, duration and type of surgery, and diagnosis were equally distributed among patients with and patients without DVT. A primary or metastatic brain tumor was present in 70% of the patients with DVT and in 84% of patients without DVT. There were no significant differences in baseline values of nAPC-SR, factor V:C, factor VIII:C, and PC between the two groups.

Perioperative nAPC-SR values in both groups are shown in Fig. 1. A lowered nAPC-SR was observed prior to surgery in 7 (70%) of 10 patients who developed DVT and in 24 (77%) of 31 patients without DVT (*p* = .68). After surgery, nAPC-SR decreased and was lowered in all patients (100%) of the DVT group, compared with 23 (74%) and 26 (84%) of patients without DVT at 3 and 7 days after surgery, respectively.

The levels of factor V:C (*p* = .003), factor VIII:C (*p* = .0002), and PC (*p* = .03) increased significantly after surgery (Table 2). Changes of factor VIII:C levels were most pronounced. No differences were demonstrated between patients with and patients without DVT.

TABLE 1. Baseline characteristics of 41 patients

	DVT	No DVT	p value
Gender (M/F)	2/8	20/11	.03
Age (years)	52.0 ± 8.6	46.3 ± 14.1	.24
QI (kg/m <sup>2</sup> )	26.8 ± 2.9	23.7 ± 3.2	.01
Duration of surgery (minutes)	336 ± 117	318 ± 113	.65
Type of surgery (no.)			
Craniotomy			
tumor/metastasis	7	26	.38
clipping aneurysm	2	1	.14
subarachnoidal bleeding	0	1	1.00
Miscellaneous*	1	3	1.00
nAPC-SR (0.82–1.22)†	0.76 (0.64–0.91)	0.74 (0.50–1.01)	.94
Factor V:C (50–150%)†	105 (84–135)	117 (87–153)	.24
Factor VIII:C (50–150%)†	134 (117–280)	168 (47–408)	.76
PC (63–138%)†	122 (87–175)	119 (85–205)	1.00

QI, Quetelet index; DVT, deep vein thrombosis; nAPC-SR, normalized activated protein C sensitivity ratio; factor V:C, factor V clotting activity; factor VIII:C, factor VIII clotting activity; PC, protein C antigen. Mean ± SD is given for age, QI and duration of surgery. Median (range) is given for nAPC-SR, factor V:C, factor VIII:C, and PC.

\* Includes decompression of the trigeminal nerve and pituitary tumor surgery.

† Normal range.

Logistic regression analysis showed no relation between the level of nAPC-SR and the development of DVT ( $p = .93$ ,  $.47$ , and  $.47$  for nAPC-SR before surgery, and at 3 and 7 days after surgery, respectively). Female gender and the Quetelet index ( $\geq 25$  kg/m<sup>2</sup>) were identified as independent predictors of DVT; odds ratios (95% confidence interval) were 12.0 (1.5–100.0;  $p = .02$ ) for women versus men and 17.8 (2.3–140.4;  $p = .006$ ) for a Quetelet index  $\geq 25$  kg/m<sup>2</sup> versus  $< 25$  kg/m<sup>2</sup>. An interaction between gender and excess weight could not be assessed because of the small number of patients. However, six of eight overweight women developed DVT and only two of eight overweight men. In contrast, 2 of 11 normal weight women developed DVT and none of 14 normal weight men.

Multiple regression analysis showed an inverse relation between nAPC-SR and factor VIII:C (regression co-

efficient =  $-0.0004$ ,  $p = .0003$ ) and between nAPC-SR and PC (regression coefficient =  $-0.0008$ ,  $p = .02$ ). Women had a lower nAPC-SR than men (regression coefficient =  $-0.07$ ,  $p = .0002$ ). The nAPC-SR was not related to age, Quetelet index, diagnosis, and factor V:C levels.

## DISCUSSION

This study showed a decreased sensitivity to APC in 80% of neurosurgical patients at 3 days, and in 88% at 7 days after surgery. Remarkably, 76% of the patients already had a lowered nAPC-SR before surgery. Although DNA analysis was not done, this finding cannot be explained by the factor V:Q<sup>506</sup> mutation, as its prevalence in the Dutch population is approximately 5% (3,4). Probably  $< 5\%$  of patients carried this mutation, since patients with a history of DVT had been excluded from the trial. Therefore, APC resistance as observed in a majority of the patients probably was acquired. The lowered nAPC-SR was not due to oral contraceptives or pregnancy, which are known to be associated with a decreased sensitivity to APC (5–8). A relation with a preoperative condition seems likely because most patients exhibited APC resistance 1 day before surgery. Of clinical baseline characteristics, only sex influenced the measured values of nAPC-SR according to previous reports (5,7). Levels of factor VIII:C and PC were inversely related to nAPC-SR. A similar relationship between factor VIII:C and nAPC-SR has been observed in women who used oral contraceptives and in pregnant women (6,12). Elevated baseline levels of factor VIII:C might be due to the disorders for which surgery was performed, mainly brain tumors and cerebrovascular events or malformations. The routinely applied perioperative dexamethasone therapy in a majority of patients might be an alter-

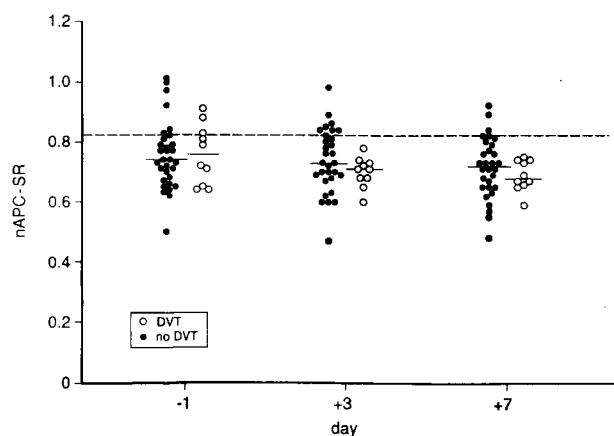


FIG. 1. Course of nAPC-SR on the day prior to surgery (–1), day 3 (+3), and day 7 (+7) after surgery, in patients with (○) and without (●) deep vein thrombosis (DVT). Median values are indicated per group. Horizontal line denotes the lower limit of normal. nAPC-SR, normalized APC sensitivity ratio.

**TABLE 2.** Perioperative values of factor V clotting activity (factor V:C), factor VIII clotting activity (factor VIII:C) and protein C antigen (PC) on the day prior to surgery (-1) and day 3 (+3) and day 7 (+7) after surgery

		Day -1	Day +3	Day +7	p†
Factor V:C	DVT	105 (84-135)	123 (81-156)	120 (96-132)	0.003
(50-150%)*	No DVT	117 (87-153)	126 (66-171)	128 (86-192)	
Factor VIII:C	DVT	134 (117-280)	297 (132-390)	234 (146-423)	0.0002
(50-150%)*	No DVT	168 (47-408)	260 (110-465)	224 (100-450)	
PC	DVT	122 (87-175)	134 (98-179)	145 (114-170)	0.03
(63-138%)*	No DVT	119 (85-205)	122 (67-180)	132 (88-200)	

Median and range are given. \*Normal range. †Statistical significance of overall effect in time. DVT, deep vein thrombosis.

native explanation. Although this effect of dexamethasone has not been reported before, increasing levels of factor VIII:C have been demonstrated in patients who received prednisone (13). The less pronounced elevated baseline PC levels in our patients might similarly be attributed to this drug (14). Furthermore, elevated PC levels have been reported in patients with glioblastoma or brain metastases, as compared with other brain tumors (15). However, the former tumors were present in only four of our patients (baseline PC levels 136-180%).

After surgery factor VIII:C levels significantly increased, especially in patients who developed DVT. Levels of factor V:C and PC showed a significant, but slight increase. Thus, the observed change in nAPC-SR may have been caused mainly by an incline of factor VIII:C. Other studies (16,17) showed increasing levels of factor VIII:C after surgery, whereas factor V:C levels did not change and PC levels commonly decreased with a nadir at the second postoperative day. Variation in the postoperative course of factor V:C and PC levels can be explained by a difference in patient handling. Neurosurgical patients are often made slightly hypovolemic and hyperosmolar to minimize cerebral edema, while ample fluid infusion is given in other types of surgery. Hemocoagulation might have resulted in the observed slight increase of factor V:C and PC.

Changes in levels of other coagulation and anticoagulant factors that are determinants of the aPTT may also have contributed to the observed decrease in sensitivity to APC. Elevated levels of factor IX, for example, have been demonstrated after surgery for brain tumors (18). We measured levels of factor V:C, factor VIII:C, and PC, considering that an imbalance of these proteins might result in APC resistance, as they are involved in the protein C anticoagulant pathway. Protein S, though involved in this pathway too, was not taken into account because its influence on APC ratio remains limited to levels <20% (4). Whether acquired APC resistance is explained sufficiently by changing levels of the mentioned factors, particularly factor VIII, remains questionable. In a recent study (8), acquired APC resistance due to oral contraceptives was demonstrated by measuring

the effect of APC on thrombin formation. In the latter test, thrombin generation is initiated via the extrinsic pathway of coagulation (19), whereas in aPTT-based test systems coagulation is initiated via the intrinsic coagulation pathway. Irrespective of its mechanism, a decreased sensitivity to APC, as demonstrated by different tests and in various conditions recognized as thrombotic risk factors, supports the concept of acquired APC resistance as a prothrombotic state.

It seems plausible that acquired APC resistance contributes to venous thromboembolism analogous to its hereditary counterpart. Of nAPC-SR values in the presented patients, 81% (preoperatively) to 88% (postoperatively) were in the same range as we found in heterozygous carriers of the factor V:Q<sup>506</sup> mutation (0.51-0.83). A lowered nAPC-SR was not identified as a risk factor for postoperative DVT, demonstrated by venography in 30% of patients with a normal and in 23% of those with a lowered sensitivity to APC.

The small number of the patients is a limitation of this study. Nevertheless, the results are interesting because it is the first study addressed to the role of acquired APC resistance in the development of DVT. Moreover, all patients were scheduled prospectively for venography and they did not receive thromboprophylactic drugs. Our findings emphasize the need for properly designed and adequately powered clinical studies to establish the clinical relevance of acquired APC resistance. This also regards other thrombotic risk factors that have been associated with acquired APC resistance, such as the use of oral contraceptives and pregnancy (5-7). Recommendations for clinical practice, based on the supposed causal relationship of a lower sensitivity to APC with a higher thrombotic risk of third-generation versus second-generation oral contraceptives (8,9), are premature in our opinion until convincing supportive evidence becomes available.

Our findings show that the applied test to measure nAPC-SR is not suitable to demonstrate hereditary APC resistance in neurosurgical patients who exhibit venous thromboembolism. A modification by predilution of the test plasma with factor V deficient plasma is one of the

alternatives for DNA analysis to reliably identify carriers of the factor V:Q<sup>506</sup> mutation (20).

### CONCLUSION

This study showed that acquired APC resistance is commonly found in neurosurgical patients who are at high risk of DVT. However, this in vitro phenomenon was not related to the development of DVT. Its clinical relevance in conditions associated with an increased risk of venous thromboembolism remains to be established.

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